Check for updates

ORIGINAL ARTICLE

Clinical characterisation and long-term survival of paediatric and juvenile lymphoma in cats: 33 cases (2008-2022)

F. Rogato *,[1], J. B. Tanis[†], B. Pons Gil[‡§], C. Pittaway[¶], C. A. Johnston[**] and A. Guillén *

*Department of Clinical Sciences and Services, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, AL9 7TA, UK
[†]Institute of Systems, Molecular and Integrative Biology, Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, L69 7BE, UK
[‡]Southfields Veterinary Specialists, Basildon, SS14 3AP, UK
[§]Hospital Veterinario Anicura Aitana, Valencia, 46920, Spain
[¶]Dick White Referrals, Six Mile Bottom, CB8 0UH, UK
**Southern Counties Veterinary Specialists, Ringwood, BH24 3JW, UK

[1]Corresponding author email: frogato20@rvc.ac.uk

Objectives: The aims of this study were to describe the clinical presentation, tumour characteristics, responses to chemotherapy protocols and toxicity in a cohort of cats with lymphoma up to 18 months of age. In addition, the probability of long-term (>2 years) survival was explored.

Materials and Methods: The medical records of client-owned cats aged up to 18 months diagnosed with lymphoma between 2008 and 2022 at five UK-based veterinary referral hospitals were reviewed.

Results: Thirty-three cats were included. The most common anatomical forms were mediastinal (42%), disseminated disease (30%) and renal (15%), with all cats having intermediate to large cell lymphoma. Three out of 29 cats tested were positive for FeLV but none for FIV. Twenty-six cats were treated with multi-agent chemotherapy protocols with complete and partial responses seen in 46% and 50% of cats, respectively. For this group, median progression-free survival was 133 days (95% confidence interval [CI] 67 to 199) and median survival time was 268 days (95% CI 106 to 430). Complete response to chemotherapy was associated with a longer progression-free survival. Seven cats were considered long-term survivors (>2 years). Chemotherapy was generally well tolerated with none of the long-term survivors suffering from chronic sequelae from cytotoxic treatment.

Clinical Significance: Paediatric and juvenile cats with lymphoma showed a high response rate to multi-agent chemotherapy protocols with rare significant toxicities. The presence of long-term survivors may suggest a more favourable outcome in a subset of patients.

Journal of Small Animal Practice (2023); **64**, 788–796
DOI: 10.1111/jsap.13667

Accepted: 12 July 2023; Published online: 11 August 2023

INTRODUCTION

Lymphoma is the most common malignant neoplasia in cats, accounting for 50% to 90% of all haematopoietic tumours in this species with an estimated incidence in the United Kingdom (UK) in 2016 of 32 per 100.000 cats (Valli *et al.* 2000, Economu *et al.* 2021). Although most lymphomas typically present in older domestic crossbreed cats (median age at diagnosis of 10 years),

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

a bimodal distribution in occurrence has been reported with a first peak seen at a young age (<24 months; Gabor *et al.* 1998). Retroviral infections are a known risk factor, with feline leukaemia virus (FeLV) infection increasing the odds of developing this cancer over 60 times (Shelton *et al.* 1990). In particular, a strong association has been reported between FeLV and the mediastinal and peripheral nodal anatomical presentations in young cats (Hardy Jr. *et al.* 1977, Francis *et al.* 1979, Shelton *et al.* 1990, Vail *et al.* 1998, Louwerens *et al.* 2005).

Since the development of diagnostic assays and the widespread introduction of vaccination against FeLV, the prevalence of FeLV infection has drastically reduced with more recent reports showing an increased incidence of intestinal lymphoma and an older age at presentation (Louwerens *et al.* 2005, Economu *et al.* 2021). Despite a decrease in the prevalence of FeLV, haematopoietic malignancies are currently the most common cancers in paediatric cats. Schmidt *et al.* (2010) reported that lymphoma accounted for 22% of all histopathological samples of tumours submitted from cats aged less than 12 months; however, retroviral status was not provided. This advocates the possibility of additional contributing factors, such as environmental and heritable influences (Gabor *et al.* 1998, Bertone *et al.* 2002, Teske *et al.* 2002, Louwerens *et al.* 2005, Schmidt *et al.* 2010, Fabrizio *et al.* 2014).

Due to the anatomical, histopathological and molecular heterogenicity of the disease, there is no consensus on the best treatment approach for feline lymphoma. However, for large-cell lymphomas, moderate to high response rates (60% to 85%) have been reported with COP (cyclophosphamide-vincristine-prednisone) and CHOP-based (cyclophosphamide-doxorubicin-vincristine-prednisone) chemotherapy protocols with a subset of patients achieving long-term survival (Teske *et al.* 2002, Taylor *et al.* 2009, Fabrizio *et al.* 2014, Rodriguez-Piza *et al.* 2023). Interestingly, various age-related differences in tumour biology and survival are seen in people with non-Hodgkin lymphoma (NHL); while children generally present with high-grade lymphomas, they tend to have a better prognosis compared to older patients with a 5-year survival rate of 87% (Crom 1994, Pfreundschuh *et al.* 2004, Sandlund 2015, Sandlund & Martin 2016). Although biological differences between children and adults have been described among NHL histological subtypes, age-related pharmacokinetic factors may also impact prognosis: the maximum tolerated dose for many chemotherapy agents is higher in younger patients, which has also been associated with improved outcomes (Crom 1994, Pfreundschuh *et al.* 2004). Nevertheless, young NHL survivors may have long-term side effects from chemotherapy, such as the development of a second neoplasm or a chronic health condition (Leung *et al.* 2001, Suh *et al.* 2020).

Clinical presentation, disease characteristics, responses to treatment and prognosis have been scarcely investigated in paediatric and juvenile FeLV-negative cats. This is particularly important for owners who may face ethical dilemmas at the time of deciding whether to proceed with intensive treatments in a young cat if the prognosis is uncertain or poor. Paediatric cancer patients may also present other challenges: it is unknown how the administration of cytotoxic drugs could affect the cat's development and the differences in drug metabolism and clearance may make accurate dosing difficult.

The aims of this study were to describe the clinical presentation, tumour characteristics, chemotherapy-associated responses and toxicity in a cohort of cats with lymphoma up to 18 months of age. The secondary aim was to investigate the outcome and probability of long-term (longer than 2 years) survival.

MATERIALS AND METHODS

Case selection and data collection

This was a multi-institutional, retrospective, descriptive case series. The electronic medical records of five UK-based veterinary referral hospitals were retrospectively reviewed to include client-owned cats aged up to 18 months diagnosed with lymphoma between March 2008 and April 2022. The medical records systems were searched using the terms "lymphoma" and "LSA" and, when possible, the age restriction tool was used to search patients within the age range of interest. Data was retrieved by a single operator (author) for each centre and was then manually reviewed to identify cats that had a confirmed diagnosis of lymphoma by cytology and/or histology and that were aged up to 18 months when the diagnosis was made. This age cut off was chosen because it is when complete closure of the growth plates and skeletal maturation occur (Miranda *et al.* 2020). Cats were excluded if the diagnosis of lymphoma could not be confirmed and if clinical records were incomplete or not available for review. Cats diagnosed at necropsy that had not received cytotoxic treatment but fulfilled the above criteria were included. Data regarding signalment, presenting clinical signs, tumour anatomical location, retroviral status, diagnostic tests including imaging investigations and disease extent, immunophenotype, toxicity and response to first-line chemotherapy protocols, chemotherapy doses, rescue chemotherapy protocols and survival were obtained and reviewed.

Haematological and biochemical abnormalities were recorded based on the reference ranges used by the individual analyser or laboratory. Cytology and histopathology specimens were assessed by board-certified pathologists or pathology trainees under supervision at the time of diagnosis, and reports were subsequently reviewed. Lymphoma cell size was classified as small if neoplastic lymphocytes had a nucleus smaller than two red blood cells (RBCs), intermediate size if the nucleus was two to three times the size of an RBC and large cell if the nucleus was greater than three times an RBC (Gambini *et al.* 2021). An anatomical classification was provided according to predominant location of the tumour burden based on physical examination, imaging investigations and cytological or histopathological diagnosis. When a single category could not be assigned, the lymphoma was classified as "disseminated disease". Abnormalities on imaging that were not sampled were presumed to be lymphoma-related based on the imaging reports, clinical presentation and the primary clinician's judgment.

Responses to treatment were assessed for multi-agent chemotherapy protocols COP, COAP (COP+cytarabine), L-COP

17485827, 2023, 12, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jsap.13667 by Royal Veterinary College, Wiley Online Library on [18/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(COP+L-asparaginase), CEOP (COP+epirubicin), L-CEOP (CEOP+L-asparaginase) and CHOP-based protocols.

The best response to treatment was recorded for cats receiving multi-agent chemotherapy protocols. Response was considered complete (CR) if there was 100% reduction in size of all clinically detectable tumour by palpation or by diagnostic imaging when needed and partial (PR) if there was ⩾50% but <100% reduction in tumour size. No change in size, ⩽50% reduction in size and increase in size of all measurable disease was classified as no response (NR). Objective response rate (ORR) was defined as the proportion of complete and partial responders (Teske *et al*. 2002, Simon *et al*. 2008). Cats that died or were euthanased within 10 days of starting chemotherapy regardless of cause were not considered as responders. All toxicity events from chemotherapy for each cat were graded based on the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (VCOG-CTCAE criteria) and was assessed only for the first-line protocol (2016). In an attempt to investigate possible long-term side effects from chemotherapy, the development of stunted growth, chronic health conditions, which included any inflammatory or immune-mediated disease that required medical intervention for longer than 3 months, and second cancers during the follow-up time were also recorded. Follow-up information was obtained from medical records and/or telephone conversation with referring veterinarians. Due to the retrospective and multi-institutional nature of the study the imaging modality and frequency of clinical restaging were not standardised and were performed at the attending clinician's discretion. Cats that received chemotherapy and were alive after 2 years from diagnosis were considered long-term survivors.

Statistical analysis

Categorical data were described as frequency and percentages. Continuous data were tested for normality. If normally distributed, the data were summarised as mean and standard deviation. If non-normally distributed, the data were summarised using median and range. Categorical variables were compared using the chi-squared or Fisher's exact tests as appropriate (Kirkwood & Sterne 2010). The Kaplan–Meier product limit analysis and the log-rank test were used for survival analysis. Progression-free survival (PFS) was defined as time from date of chemotherapy initiation to date of disease progression (based on physical examination, cytology, histopathology or imaging). Survival time (ST) was calculated from the date of diagnosis to the date of death or lost to follow-up. Cats that were lost to follow-up were considered to be dead from the disease at the time of last contact. Cats that received single-agent chemotherapy protocols, that died of unrelated causes or were still alive at the end of the study were censored from ST assessment. Cats that died of unrelated causes were included in the PFS assessment, but cats that received single-agent chemotherapy protocols or were still alive at the end of the study were censored. The log-rank test was used to evaluate chemotherapy and anatomical forms as variables for influence on STs. A P value <0.05 was considered significant. All statistical analysis was performed by SPSS statistic software version 27.0.

Ethics approval was granted by the Social Science Research Ethical Review Board at the Royal Veterinary College (URN SR2020-0225).

RESULTS

Signalment and clinical presentation

In total, 47 cats were assessed for eligibility. Of these, 14 were excluded due to the absence of definitive diagnosis (n=8) or incomplete clinical records (n=6). Thirty-three cats were included in the study. The median age at diagnosis was 12 months (range: 3 to 18 months). Two cats (6%) aged less than 6 months, 16 cats (48%) aged between six and 12 months, and 15 cats (45%) aged between 13 and 18 months. Breeds included were Domestic Shorthair (n=15; 45%), Siamese (n=4; 12%), British Shorthair (n=3; 9%), Bengal (n=2; 6%), Russian Blue (n=2; 6%) and one each (3%) of Domestic Longhair, Domestic Medium Hair, Maine Coon, Oriental Shorthair, Savannah, Somali and crossbreed. There were 19 neutered males (57%), four spayed females (12%), five entire males (15%) and five entire females (15%). The male to female ratio was 2.7:1.0.

Presenting clinical complaints were dyspnoea (n=21; 63%), lethargy (n=16; 48%), peripheral lymphadenopathy (n=14; 42%), inappetence (n=12; 36%), weight loss (n=6; 18%), vomiting (n=4; 12%), polyuria-polydipsia (n=2; 6%) and nasal discharge (n=1; 3%). Median duration of clinical signs before presentation was 4 days (range: 0 to 60 days).

Diagnostic investigations

Haematology and serum biochemistry profiles were performed in 31 cats and urinalysis was available in eight cats. The most commonly recorded haematological abnormalities were anaemia (n=8; 26%) and lymphopenia (n=11; 33%). The most common biochemistry abnormalities were increased liver enzymes' activity (ALT only n=1; ALP only n=2; or both n=2; 16%) and increased renal parameters (urea only n=4; both urea and creatinine n=5; or SDMA n=2; 32%). Twenty-nine cats had retroviral tests results available: three tested positive for FeLV antigenaemia and none tested positive for FIV antibodies.

Thoracic and abdominal radiographs were performed in 18 cats and in four cats, respectively. CT scan of the thorax and abdomen was performed in five cats. Thoracic, abdominal, ocular ultrasound and echocardiography were performed in 13, 21, one and two cats, respectively. Overall, 18 cats had both thoracic and abdominal imaging performed. Cytological samples were obtained from 32 cats and additional surgical biopsies were obtained from four cats, including laparoscopic liver biopsies in one cat with hepatic lymphoma, lymphadenectomy of peripheral lymph node in two cats with disseminated lymphoma and core-needle biopsies of the kidney in one cat with renal lymphoma. Complete cytological description was available in 29 cats: all cats were reported to have intermediate to large cell lymphoma. Cell morphology was described as intermediate (n=2), intermediate to large (n=20), large (n=6), and large with Mott cell differentiation (n=1). In one cat cytology of a peripheral lymph node was

17485827, 2023, 12, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jsap.13667 by Royal Veterinary College, Wiley Online Library on [18/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

not conclusive and bone marrow core biopsies were required for diagnosis and in another cat the diagnosis was achieved following *post-mortem* examination. Histological grade was available in three cats, two were high grade and one intermediate grade. No small cell lymphomas were observed. Flow-cytometry was performed in 10 cats and polymerase chain reaction (PCR) for antigen receptor rearrangements (PARR) was concurrently performed in two cats. From those, three cats had B-cell and seven cats had T-cell immunophenotype.

Based on clinical presentation and staging results, the most common anatomical forms were mediastinal (14 cats; 42%) and disseminated disease (10 cats; 30%), followed by renal (five cats; 15%), peripheral multi-nodal, ocular, intestinal and hepatic (one each; 3%) (Figs 1 and 2). Of the cats with disseminated disease, six cats presented with peripheral multi-nodal involvement and multi-organ bicavitary involvement, three cats presented with multi-organ bicavitary involvement and one cat presented with peripheral multi-nodal involvement and mediastinal involvement (Table 1). For cats with renal lymphoma, no neurological signs were reported at presentation. Of the three cats that tested positive for FeLV antigenaemia, two had the mediastinal form and one had disseminated disease.

Table 1. Summary of the main radiographic, ultrasonographic and CT scan findings associated with mediastinal, disseminated and renal lymphoma in paediatric and juvenile cats

	n
Mediastinal lymphoma (n=14)	
Pleural effusion	14
Mediastinal mass/lymphadenopathy	14
Disseminated lymphoma (n=10)	
Peripheral lymphadenopathy†	6
Mediastinal mass/lymphadenopathy	10
Pleural effusion	10
Hepatosplenomegaly	2
Abdominal lymphadenopathy	9
Peritoneal effusion	2
Renal lymphoma (n=5)	
Bilateral renomegaly	5
Retroperitoneal effusion	5

†Also identified in eight additional cats based on palpation of lymph nodes

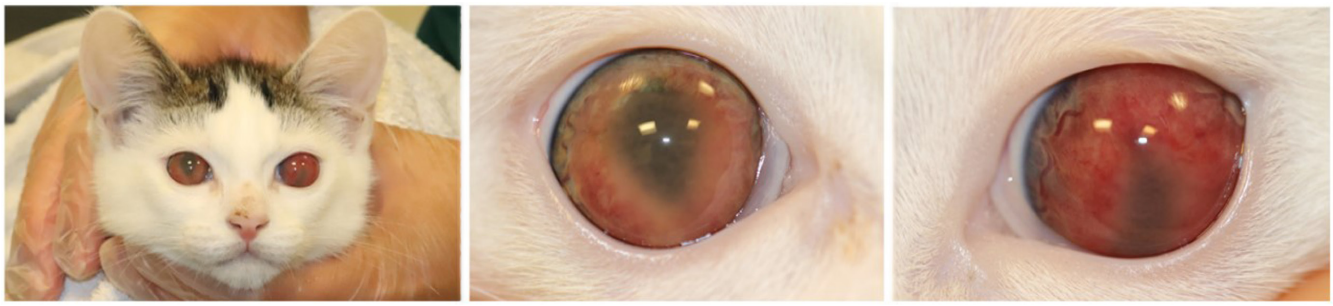

FIG 1. A 3-month old, male entire, Domestic Shorthair was presented for uveitis. Ophthalmic examination of both eyes revealed corneal oedema, a markedly hyperaemicand swollen iris, dyscoria, moderate aqueous flare and presence of a large fibrin clot in the anterior chamber. Cytopathologic diagnosis of aqueous humour was consistent with large cell lymphoma

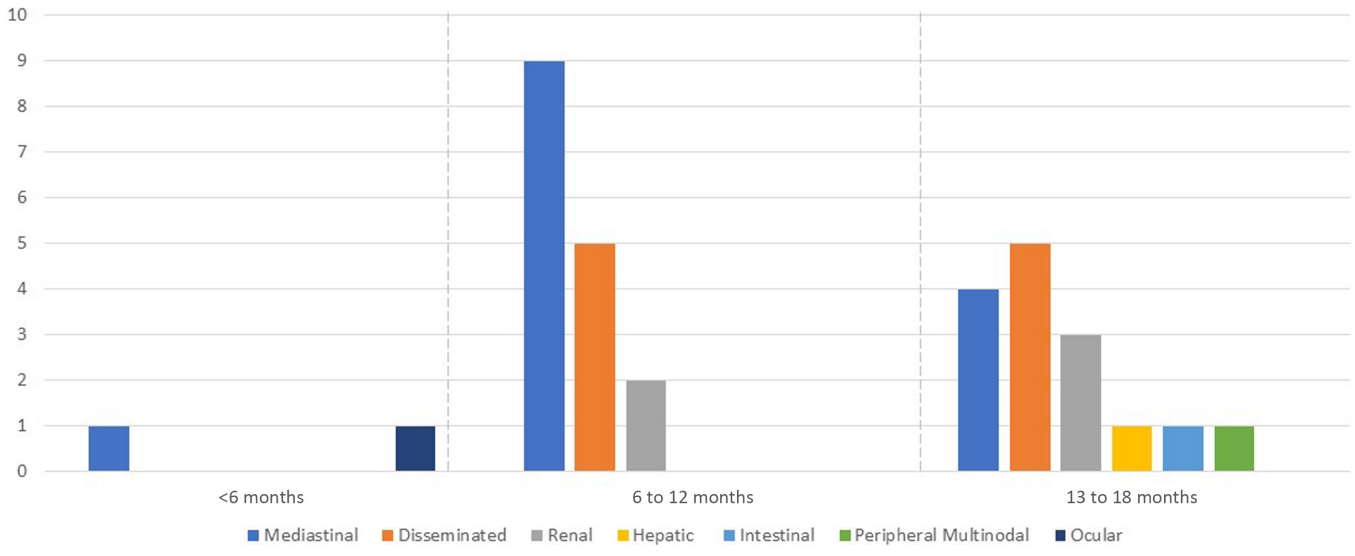

FIG 2. Bar chart showing the age distribution in relation to the anatomical form of lymphoma in 33 paediatric and juvenile cats

17485827, 2023, 12, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jsap.13667 by Royal Veterinary College, Wiley Online Library on [18/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Treatment and adverse events

Twenty-eight cats received chemotherapy with 26 of them receiving a multi-agent chemotherapy protocol as first-line therapy (Table 2). Twelve cats received a COP protocol, three cats received CHOP, five cats received a COAP protocol, three cats received a COP protocol with L-asparaginase (L-COP), one cat received a CHOP protocol with cytarabine, one cat received a CEOP protocol with L-asparaginase (L-CEOP). One cat was treated with lomustine followed by L-COP protocol. Doses were recorded for the different chemotherapy agents including vincristine (dose range: 0.35 to 0.7 mg/m^2), cyclophosphamide (dose range: 127 to 250 mg/m^2), doxorubicin/epirubicin (dose: 1 mg/kg), cytarabine (dose range: 250-300 mg/m^2) and L-asparaginase (dose: 400 IU/kg or 10,000 IU/m^2). One cat received lomustine with L-asparaginase and one cat received vinblastine alone. Two cats had received corticosteroids before presentation. Eleven cats completed the first-line protocol, eight cats were transitioned to a rescue protocol and in three cats it was discontinued due to chemotherapy-related toxicities. One cat was still receiving the first-line protocol at the time of writing the manuscript. Information regarding the remaining five cats was unavailable.

After completing a multi-agent chemotherapy protocol, six cats received maintenance chemotherapy with a chlorambucil-prednisolone protocol. Thirteen cats received second-line rescue protocols, and six of these cats then received third-line rescue protocols. Second-line chemotherapy protocols included single-agent lomustine (n=4), L-asparaginase and epirubicin (n=1), doxorubicin and cyclophosphamide (n=1), vincristine and lomustine (n=1), L-asparaginase, doxorubicin and lomustine (n=1), epirubicin, vincristine and cyclophosphamide (n=1), epirubicin only (n=1), cytarabine and lomustine (n=1), and transition from COP to CEOP protocol (n=1). Third-line chemotherapy protocols included single-agent doxorubicin (n=2), L-asparaginase and lomustine (n=1), lomustine single agent (n=3) and L-asparaginase only (n=1).

Table 2. Age distribution, anatomical classification, and responses of 28 paediatric and juvenile cats with large cell lymphoma treated with chemotherapy

	No. cats	Response (CR/PR)	No response (SD or PD)	Alive >2 years
Age				
<6 months	0	–	–	0
6 to 12 months	16†‡	14	1	4
13 to 18 months	12	12	0	3
Anatomical classification				
Mediastinal	12‡§	4/6	1	2
Disseminated	10†	3/7	0	1
Renal	5	4/1	0	3
Hepatic	1	1/0	0	1
Multi-agent chemotherapy protocols				
L-COP	4¶	2/1	1	1
COP	12	4/8	0	2
COAP	5	3/2	0	2
CHOP	3	1/2	0	0
L-CEOP	1	1/0	0	1
CHOAP	1	1/0	0	1
Total	26	25	1	7
Other chemotherapy				
L-asparaginase and lomustine	1	–	–	0
Vinblastine	1	1	0	0

†One cat received vinblastine
‡Response could not be evaluated in one cat
§One cat received L-asparaginase and lomustine
¶One cat had lomustine before L-COP

Toxicity to first-line protocols could be assessed in 27 cats. Eleven cats suffered from grade I chemotherapy-related toxicities, including neutropenia (n=7), diarrhoea (n=3) and inappetence (n=1). Four cats suffered from grade II toxicities, including neutropenia (n=1), thrombocytopenia (n=1) and inappetence (n=2). One cat suffered from grade III and one from grade IV neutropenia. As a result, treatment delays occurred in three cats and dose reductions in four cats. No cats required hospitalisation due to chemotherapy-related toxicities.

Treatment response and progression-free survival

Treatment response to first-line multi-agent chemotherapy protocols was available for the 26 cats. Twelve cats achieved CR (46%), 13 cats achieved PR (50%) and one cat was a NR with an ORR of 96%. The median PFS for responders was 133 days (95% confidence interval [CI] 67 to 199). The median PFS for cats that achieved CR was 868 days (95% CI 0 to 1976) and for those that achieved PR was 63 days (95% CI 39 to 87). This difference was statistically significant (P=0.001) (Fig 3). Cats with renal lymphoma had numerically longer PFS (1092 days, 95% CI 0 to 2457) than cats with disseminated lymphoma (70 days, 95% CI 0 to 151) and mediastinal (70 days, 95% CI 0 to 170) anatomical forms, but the small group numbers did not allow for statistical comparison.

Outcome and survival analysis

One cat with renal and one cat with mediastinal lymphoma were still alive at the time of writing the manuscript with a follow-up of 65 and 51 days, respectively. Twenty-six cats died or were considered dead due to lymphoma and five died due to causes unlikely related to lymphoma. Causes of death unlikely related to lymphoma included urethral obstruction, hypoglycaemia together with gastroenteritis, acute kidney injury and ataxia, trauma and suspected septic shock. The median survival time (MST) for cats treated with multi-agent chemotherapy protocols as first-line therapy was 268 days (95% CI 106 to 430). The MST for cats that achieved CR was 858 days (95% CI 27 to 1688) and for those that achieved PR was 150 days (95% CI 0 to 307); however, this difference was not statistically significant (P=0.09).

The MST was 180 days (95% CI 92 to 268), 210 days (95% CI 67 to 353) and 1482 days (95% CI 140 to 2823) for cats with mediastinal, disseminated disease and renal form treated with multi-agent chemotherapy protocols, respectively.

Long-term survival

The overall 1-, 2- and 3-year survival probability for cats treated with chemotherapy was 25%, 25% and 14%. Seven cats were considered long-term survivors with survivals of over 2 years (MST 1569 days; 95% CI 1346 to 1792). Of these, one cat had hepatic lymphoma, two cats had mediastinal lymphoma, one cat had disseminated disease and three cats had renal lymphoma

17485827, 2023, 12, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jsap.13667 by Royal Veterinary College, Wiley Online Library on [18/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

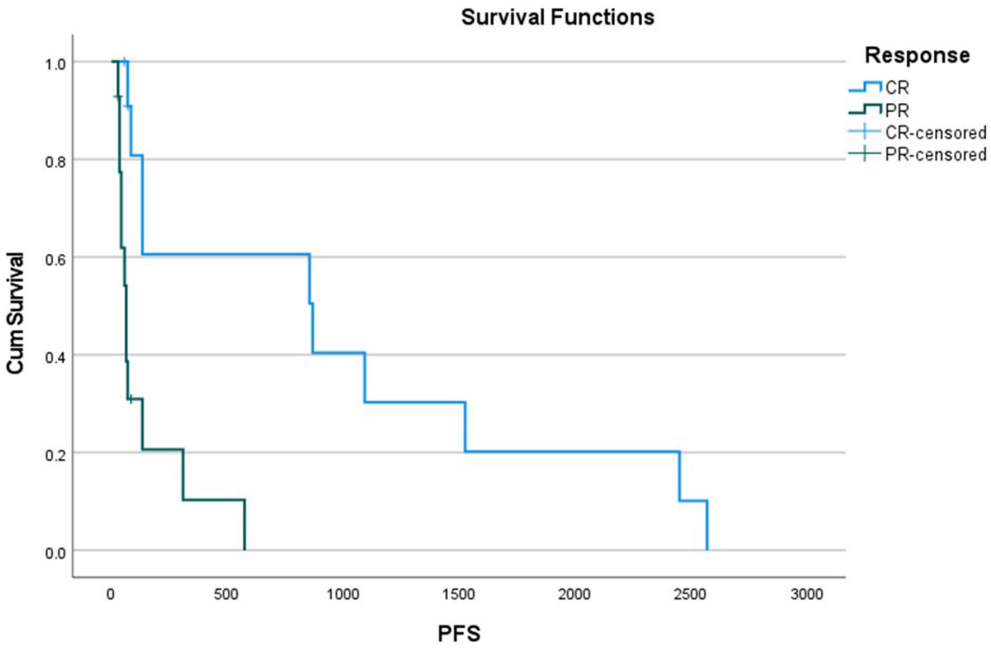

FIG 3. Kaplan–Meier curve showing the progression-free survival (PFS) for cats that responded to first-line multi-agent chemotherapy protocols. The median PFS for cats that achieved complete response was significantly longer than for cats that achieved partial response (868 *versus* 63 days, P=0.001)

Table 3. Signalment, anatomical classification, chemotherapy protocols and responses of paediatric and juvenile cats with lymphoma treated with chemotherapy and achieving long-term survivals (>2 years)

N°	Age (months)	Breed	Anatomical form	Histological grade	Chemotherapy protocol	Response	MST (days)
1	16	Bengal	Renal	–	COAP	CR	1482
2	12	Savannah	Renal	–	COAP	CR	1569
3	18	Russian Blue	Renal	Intermediate	CHOAP	CR	2570
4	12	Siamese	Mediastinal	–	CEOP	CR	2687
5	11	British Blue	Mediastinal	–	COP	PR	2573
6	6	Siamese	Disseminated	–	COP	CR	858
7	17	DSH	Hepatic	–	L-COP	CR	868

DSH Domestic short hair, CR Complete response, PR Partial response, MST Median survival time

(Table 3). All these cats tested negative for FeLV antigenaemia and FIV antibodies.

For those cats, no signs of stunted growth, chronic health conditions or development of different neoplastic conditions were recorded within the time of follow-up (median follow-up time 1569 days; range 858 to 2573 days).

DISCUSSION

This study aimed to characterise paediatric and juvenile lymphoma in cats, its relationship with retroviral status and the short and long-term side effects and outcome after receiving chemotherapy.

As previously observed in other studies, males appeared to be overrepresented in this cohort of cats (Louwerens *et al.* 2005, Fabrizio *et al.* 2014, Economu *et al.* 2021). Differences in frequency of anatomical forms have been previously reported mostly depending on the prevalence of FeLV infection and genetic variations of the specific study population, with studies conducted in the 1980s showing high prevalence (75% to 85%) of FeLV infection in cats with mediastinal lymphoma (Hardy Jr. *et al.* 1977, Francis *et al.* 1979, Mooney & Hayes 1986, Shelton *et al.* 1990, Louwerens *et al.* 2005). The prevalence of the FeLV infection in our population was low (10% of the cats tested) and similar to other post-FeLV era publications in Europe and North America (Louwerens *et al.* 2005, Taylor *et al.* 2009, Fabrizio *et al.* 2014). Although PCR test for FeLV was not performed routinely to confirm retroviral status, our results may indicate that genetic or environmental factors with different pathophysiological mechanisms compared to retroviral infection could favour the development of lymphoma in young FeLV-negative cats (Gabor *et al.* 1998, Louwerens *et al.* 2005, Fabrizio *et al.* 2014).

A previous histopathological study found the peripheral nodal location to be the most common form of lymphoma in cats aged less than 12 months (Schmidt *et al.* 2010). Even though 14 cats (42%) presented with multi-nodal peripheral lymphadenopathy

17485827, 2023, 12, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jsap.13667 by Royal Veterinary College, Wiley Online Library on [18/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

in our study, 13 of them were found to have intrathoracic or abdominal involvement during imaging investigations, which highlights the importance of staging for an accurate anatomical classification. It may also be possible that the differences in anatomical distribution between studies are due to a higher proportion of cats with mediastinal lymphoma being diagnosed *via* cytology rather than histopathology (Schmidt *et al.* 2010). Intestinal lymphoma, generally affecting older cats, has been the most commonly reported anatomical form over the past three decades and unsurprisingly this form was represented by only one cat in our study.

Cytological assessment described intermediate to large lymphocyte morphology in all the samples examined in our study. This cytological feature is generally associated with high-grade lymphomas and more aggressive biological behaviour. Similar observations were reported by Schmidt *et al.* (2010) where only 10% of paediatric cats had small cell lymphoma. Histopathological assessment was available only in three cats and described as intermediate to high-grade, but the WHO classification system was not applied. Cell morphology and histopathological grade have been previously correlated to tumour topography, with mediastinal, disseminated and renal lymphoma more likely to be high-grade subtypes (Valli *et al.* 2000). Interestingly, children are also more often affected by high-grade NHLs compared to older population, albeit showing better treatment outcome than adults (Sandlund & Martin 2016).

Chemotherapy appeared to be well-tolerated with only two cats in our study experiencing severe toxicities (VCOG grade III and IV). As studies investigating pharmacokinetic and pharmacodynamic properties of chemotherapy drugs in paediatric and juvenile cats are lacking, chemotherapy agents and dosing regimens were administered at clinician's discretion and according to therapeutic ranges described for adult cats. In human medicine, recent studies have resulted in more evidence-based recommendations and guidelines, and although dosing of chemotherapy in infants and children remains to certain degree empirical, dose reductions are generally recommended at the start of treatment (Hutson *et al.* 2012, Veal *et al.* 2016, Nijstad *et al.* 2022). In our study, none of the long-term survivors had stunted growth, a second cancer or chronic disease reported within the time of follow-up. Leung *et al.* (2001) reported the incidence of developing an unrelated neoplasm in children to be 2.1% at 10 years and this to increase to 4.8% after 20 years, which has been strongly associated with the carcinogenic effects of alkylating agents and doxorubicin (Smith *et al.* 1999, Leung *et al.* 2001, Allan & Travis 2005). It may be that the lower doses of cytotoxic agents used in cats decrease the risk, but this could also have been underestimated due to the small group size. Overall, the small number of patients requiring dose reductions and low incidence of short and long-term severe toxicity would support the use of standard chemotherapy doses in paediatric and juvenile cats.

The ORR to first-line multi-agent chemotherapy protocols was high (96%); however, complete response was achieved in only 46% of the cats assessed, which is lower than previously reported in cats with mediastinal, nodal and extranodal lymphomas (Teske *et al.* 2002, Taylor *et al.* 2009, Fabrizio *et al.* 2014). Given the variety of chemotherapy protocols administered, response to specific protocols was not assessed. Even though these results may suggest a decreased probability for paediatric and juvenile cats to achieve CR, the heterogeneity of the population and treatment protocols, incomplete and non-standardised clinical staging and response assessment to chemotherapy should prompt to interpret these results with caution.

In cats treated with multi-agent chemotherapy protocols, the median PFS for responders (CR + PR) was 133 days and it was significantly longer for cats that achieved CR compared to cats only achieving PR (868 *versus* 63 days). This improved outcome associated with CR is well known in the feline lymphoma literature for many anatomical locations with various chemotherapy protocols (Teske *et al.* 2002, Fabrizio *et al.* 2014). Although response to therapy may be considered a prognostic factor, this can be assessed only after starting chemotherapy, and therefore its clinical value and importance at the time of guiding owners with decision making may be limited at diagnosis.

The MST in our study was 268 days, which is similar to the MST previously reported by Teske *et al.* (2002) of 266 days. Considering the older population studied by Teske *et al.* (2002), this result would imply a similar outcome between younger and older cats, which is different to what observed in people with an overall improved outcome in children compared to adults. According to Fabrizio *et al.* (2014), young cats with mediastinal lymphoma achieving CR lived much longer (MST of 980 days) than previously reported. However, in our study the MST cats with mediastinal lymphoma were only 180 days, which is lower compared to Fabrizio *et al.* (2014), who reported an MST of all of cats of over a year (373 days). The differences between the study populations and heterogeneity of chemotherapy protocols administered render difficult to compare results of the two studies. Despite that, the overall CR rate achieved for cats with mediastinal lymphoma in our study was of only 36%, which is lower to the response rates described by Fabrizio *et al.* (2014) for either the COP or CHOP protocols (61.5% and 66.7%, respectively). The reasons for the lower response rate in our study remain elusive; however, these likely influenced the STs. In our study, cats with peripheral and internal multi-nodal and extra-nodal involvement were classified as disseminated disease. The MST for these cats was 210 days, which is comparable to what is reported by Taylor *et al.* (2009) for cats with extra-nodal lymphoma treated with COP chemotherapy protocol, and slightly higher to what is reported by Teske *et al.* (2002) for cats with "miscellaneous" lymphoma. These results would again suggest similar outcome across populations of different ages. Cats suffering from renal lymphoma had the longest PFS (1092 days) and MST (1482 days) and accounted for three of the seven cats classified as long-term survivors. Although the small number of cases in each anatomical group precluded statistical comparisons, these are interesting results. Renal lymphoma is generally associated with an aggressive biological behaviour with overall short STs and risk of central nervous system progression in a subset of cats (Mooney *et al.* 1987, Williams *et al.* 2021). Prolonged STs (longer than 1 year) were reported in four out of 27 and three

17485827, 2023, 12, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jsap.13667 by Royal Veterinary College, Wiley Online Library on [18/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

out of 28 cats in the study by Mooney *et al*. (1987) and Williams *et al*. (2021), respectively. These findings supported also by our study indicate that a subset of cats with renal lymphoma may have long-term outcome.

The major limitation of this study is inherent to its retrospective and multi-institutional nature. The specific study population including younger cats may have influenced owners' commitment to therapy with a proportion of cats who were euthanased at diagnosis or soon after failing first-line chemotherapy protocol, which limited our study population size and consequently analysis of response rate, tolerability, and overall survival. Method of diagnosis, modality of clinical staging, chemotherapy protocols and monitoring were not standardised and varied greatly among and within contributing institutions with assessment of response limited to physical examination in some cases. Although cytology and histopathology reports were reviewed by the authors, the original samples were not reviewed. Histological grade was only available in few cases, with the possibility of some of the long-term survivors being affected by low-grade lymphomas. The small study population and various anatomical forms prevent reaching strong statistical conclusions. The lack of longer follow-up data may have limited the ability to detect sequelae and complications that could occur many years following chemotherapy. This is important to take into consideration when analysing and comparing response rates and STs, and results should be interpreted accordingly.

Our results show that paediatric and juvenile cats with lymphoma will often present with mediastinal, disseminated disease or renal involvement and will have a high response rate to multiagent chemotherapy protocols with generally a good tolerance. Although the presence of long-term survivors may indicate a more favourable outcome in a subset of patients, further studies are needed to identify specific genetic and prognostic biomarkers to better predict outcome.

Acknowledgements

The authors are grateful to Dr Emily Jeanes and Royal Veterinary College's ophthalmology department for providing the pictures included in Fig 1.

Author contributions

Francesco Rogato: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); project administration (lead); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Jean Benoit Tanis:** Data curation (supporting); investigation (supporting); visualization (supporting); writing – review and editing (supporting). **Begoña Pons Gil:** Data curation (supporting); investigation (supporting); visualization (supporting); writing – review and editing (supporting). **Charlie Pittaway:** Data curation (supporting); investigation (supporting); visualization (supporting); writing – review and editing (supporting). **Charlotte Anne Johnston:** Data curation (supporting); investigation (supporting); visualization (supporting); writing – review and editing (supporting). **Alexandra Guillén:** Conceptualization (equal); data curation (equal); formal analysis (supporting); investigation (supporting); project administration (equal); supervision (lead); validation (equal); visualization (equal); writing – review and editing (equal).

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References

Allan, J. M. & Travis, L. B. (2005) Mechanisms of therapy-related carcinogenesis. *Nature Reviews. Cancer* **5**, 943-955

Bertone, E. R., Snyder, L. A. & Moore, A. S. (2002) Environmental tobacco smoke and risk of malignant lymphoma in pet cats. *American Journal of Epidemiology* **156**, 268-273

Crom, W. R. (1994) Pharmacokinetics in the child. *Environmental Health Perspectives* **102**, 111-117

Economu, L., Stell, A., O'neill, D. G., *et al*. (2021) Incidence and risk factors for feline lymphoma in UK primary-care practice. *The Journal of Small Animal Practice* **62**, 97-106

Fabrizio, F., Calam, A. E., Dobson, J. M., *et al*. (2014) Feline mediastinal lymphoma: a retrospective study of signalment, retroviral status, response to chemotherapy and prognostic indicators. *Journal of Feline Medicine and Surgery* **16**, 637-644

Francis, D. P., Cotter, S. M., Hardy, W. D. Jr., *et al*. (1979) Comparison of virus-positive and virus-negative cases of feline leukemia and lymphoma. *Cancer Research* **39**, 3866-3870

Gabor, L. J., Malik, R. & Canfield, P. J. (1998) Clinical and anatomical features of lymphosarcoma in 118 cats. *Australian Veterinary Journal* **76**, 725-732

Gambini, M., Martini, V., Bernardi, S., *et al*. (2021) Cytology of feline nodal lymphoma: low interobserver agreement and variable accuracy in immunophenotype prediction. *Journal of Comparative Pathology* **184**, 1-6

Hardy, W. D. Jr., Zuckerman, E. E., Macewen, E. G., *et al*. (1977) A feline leukaemia virus- and sarcoma virus-induced tumour-specific antigen. *Nature* **270**, 249-251

Hutson, J. R., Weitzman, S., Schechter, T., *et al*. (2012) Pharmacokinetic and pharmacogenetic determinants and considerations in chemotherapy selection and dosing in infants. *Expert Opinion on Drug Metabolism & Toxicology* **8**, 709-722

Kirkwood, B. R. & Sterne, J. A. (2010) Essential Medical Statistics. Oxford, UK: John Wiley & Sons.

Leung, W., Sandlund, J. T., Hudson, M. M., *et al*. (2001) Second malignancy after treatment of childhood non-Hodgkin lymphoma. *Cancer* **92**, 1959-1966

Louwerens, M., London, C. A., Pedersen, N. C., *et al*. (2005) Feline lymphoma in the post-feline leukemia virus era. *Journal of Veterinary Internal Medicine* **19**, 329-335

Miranda, F. G., Souza, I. P., Viegas, F. M., *et al*. (2020) Radiographic study of the development of the pelvis and hip and the femorotibial joints in domestic cats. *Journal of Feline Medicine and Surgery* **22**, 476-483

Mooney, S. C. & Hayes, A. A. (1986) Lymphoma in the cat: an approach to diagnosis and management. *Semin Vet Med Surg Small Anim* **1**, 51-57

Mooney, S. C., Hayes, A. A., Matus, R. E., *et al*. (1987) Renal lymphoma in cats: 28 cases (1977-1984). *Journal of the American Veterinary Medical Association* **191**, 1473-1477

Nijstad, A. L., Barnett, S., Lalmohamed, A., *et al*. (2022) Clinical pharmacology of cytotoxic drugs in neonates and infants: providing evidence-based dosing guidance. *European Journal of Cancer* **164**, 137-154

Pfreundschuh, M., Trumper, L., Kloess, M., *et al*., German High-Grade Non-Hodgkin's Lymphoma Study, G. (2004) Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of young patients with good-prognosis (normal LDH) aggressive lymphomas: results of the NHL-B1 trial of the Dshnhl. *Blood* **104**, 626-633

Rodriguez-Piza, I., Borrego, J. F., Treggiari, E., *et al*. (2023) Clinical presentation, treatment and outcome in 23 cats with laryngeal or tracheal lymphoma. *Journal of Feline Medicine and Surgery* **25**, 1098612X221143769

Sandlund, J. T. (2015) Non-Hodgkin lymphoma in children. *Current Hematologic Malignancy Reports* **10**, 237-243

Sandlund, J. T. & Martin, M. G. (2016) Non-Hodgkin lymphoma across the pediatric and adolescent and young adult age spectrum. *Hematology. American Society of Hematology. Education Program* **2016**, 589-597

Schmidt, J. M., North, S. M., Freeman, K. P., *et al*. (2010) Feline paediatric oncology: retrospective assessment of 233 tumours from cats up to one year (1993 to 2008). *The Journal of Small Animal Practice* **51**, 306-311

Shelton, G. H., Grant, C. K., Cotter, S. M., *et al*. (1990) Feline immunodeficiency virus and feline leukemia virus infections and their relationships to lymphoid malignancies in cats: a retrospective study (1968-1988). *Journal of Acquired Immune Deficiency Syndromes* **1988**, 623-630

Simon, D., Eberle, N., Laacke-Singer, L., *et al*. (2008) Combination chemotherapy in feline lymphoma: treatment outcome, tolerability, and duration in 23 cats. *Journal of Veterinary Internal Medicine* **22**, 394-400

17485827, 2023, 12, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jsap.13667 by Royal Veterinary College, Wiley Online Library on [18/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Smith, M. A., Rubinstein, L., Anderson, J. R., *et al*. (1999) Secondary leukemia or myelodysplastic syndrome after treatment with epipodophyllotoxins. *Journal of Clinical Oncology* **17**, 569-577

Suh, E., Stratton, K. L., Leisenring, W. M., *et al*. (2020) Late mortality and chronic health conditions in long-term survivors of early-adolescent and young adult cancers: a retrospective cohort analysis from the Childhood Cancer Survivor Study. *The Lancet Oncology* **21**, 421-435

Taylor, S. S., Goodfellow, M. R., Browne, W. J., *et al*. (2009) Feline extranodal lymphoma: response to chemotherapy and survival in 110 cats. *The Journal of Small Animal Practice* **50**, 584-592

Teske, E., Van Straten, G., Van Noort, R., *et al*. (2002) Chemotherapy with cyclophosphamide, vincristine, and prednisolone (COP) in cats with malignant lymphoma: new results with an old protocol. *Journal of Veterinary Internal Medicine* **16**, 179-186

Vail, D. M., Moore, A. S., Ogilvie, G. K., *et al*. (1998) Feline lymphoma (145 cases): proliferation indices, cluster of differentiation 3 immunoreactivity, and their association with prognosis in 90 cats. *Journal of Veterinary Internal Medicine* **12**, 349-354

Valli, V. E., Jacobs, R. M., Norris, A., *et al*. (2000) The histologic classification of 602 cases of feline lymphoproliferative disease using the National Cancer Institute working formulation. *Journal of Veterinary Diagnostic Investigation* **12**, 295-306

Veal, G. J., Errington, J., Sastry, J., *et al*. (2016) Adaptive dosing of anticancer drugs in neonates: facilitating evidence-based dosing regimens. *Cancer Chemotherapy and Pharmacology* **77**, 685-692

Veterinary cooperative oncology group – common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1. (2016). *Veterinary and Comparative Oncology* **14**, 417-446

Williams, A. G., Hohenhaus, A. E. & Lamb, K. E. (2021) Incidence and treatment of feline renal lymphoma: 27 cases. *Journal of Feline Medicine and Surgery* **23**, 936-944

17485827, 2023, 12, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jsap.13667 by Royal Veterinary College, Wiley Online Library on [18/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License